Unit-3

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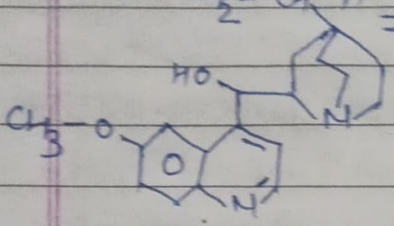
Isolation, Identification & Analysis of phytoconstituents

Alkaloids

⇓

$CH_2=CH$ # Quinine # ii. structure-



iii. Chemo-taxonomic distribution-

- Quinine & quinidine isolated from cinchona bark.
- Cinchona is the dried bark stem & root is used for the synthesis of quinine & quinidine-
- Quinine & quinidine is mainly optain from the bark, stem & roots of "Cinchona officinalis", 'Cinchona ledgeriana', 'Cinchona succirubra', 'Cinchona calisaya', family - "Rubiaceae"

- Quinine & quinidine are the stereo-isomers which comes under the categary of quinine oline alkaloids
- Quinine is leuco-rotatory-
- Quinidine is dextro-rotatory-

Uses- Quinine is an anti-malarial drugs-
- quinidine is an anti-arrhythmaic drugs-

* Quinine also consider as a better standard-

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iii) Extraction & Isolation -

- Take the dry cinchona bark & powder it with the help of grander & powder passed through #20
- mix the powder with 60% CaOH (Island like) & 40% water
- keep its for some times during the mixing slaked like fix the organic acid & Ca^{2+} salt (keep its 24 hrs)
- Take the mask & packed in a soxhleter & extract with benzene at room temp.-
- Collect the benzene extract & filter it., - after Conc. & filter (To the conc. extract & sodium carbonate soln to neutralization act → pH 6.5)

Precipitate quinine sulphate

- If coloured soln
  - Than carbon black is add.
  - filter it & Conc. the filter
  - keep its for some times quinine sulphate appearance-
- If colourless soln-
  - which is purified boiling with hot H_2O
  - Quinine sulphate & dissolve & Conc.
  - keep its for some times, crystal of quinine sulphate app.-

- Filtrate
  - To the filtrate add Na_2CO_3 & NaOH (1:1)
  - Precipitation take place know extract this soln with di-ethyl ether
  - To separate quinidix & cinchonidine-

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iv) Characterisation / Identification / Chemical test / qualitative

a) Fatt Thalleoquin test.
b) Ferrocyanide test-
c) Acid test-
d) Erythroquin test-

a) Thalleoquin test-

- To the sample add bromine water & ammonia
  ⇓
- Bright green colour fluorescence appearance.

b) Ferrocyanide test:-

- To the sample add bromine water & chloroform
  ⇓
- Shake it & allow for few minutes
  ⇓
- Add 1% potassium ferrocyanide solution & 3ml of 5M/5M NaOH solution

Quinine
⇓
- Chloroform layer turns colourless

Quinidine
⇓
- Chloroform layer turns red colour-

* R_f - value only for determine in TLC & HPLC & HPTLC in determine R_f -

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c) Acid test :-
- To the sample add dilute H_2SO_4 solution
- Sample give blue fluorescence indicate the presence of quinine.-

d) Erythroquin test-
- To the sample add dilute H_2SO_4 solution -
- To the acidic sample add bromine water & shake it well
- Add few drops of strong ammonia solution & neutralized with dilute H_2SO_4 -
- It will gives blue colouration
- Now continuous add dilute H_2SO_4, it will changes the colour from blue to red.-

v) Analysis - / Quantitative test / characterstics -

a) TLC (Thin layer chromatography)-
b) Spectromatric estimation - / HPLC -
c) HPTLC (High performance thin layer chromatography

a) TLC - i) Sample preparation
ii) Stationary phase
iii) Solvent system (mobile phase)
iv) Spring reagent (detecting reagent)-
v) R_f - value - (Retardition factor) -

*Blank - These are substances/agent in drugs analysis
(other than drugs)

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Dissolve

i) Sample preparation - 1mg of quinine in 1ml of methanol

ii) Stationary phase → Silica-gel-G (*G = Gypsum)

iii) Solvent phase/mobile phase - Chloroform : Diethylamine
(9 : 1 or 9.6 : 0.4)

iv) Spring reagent/ detecting agents -
• Dragendroff's reagent -
• modified dragendroff's reagent (Kraut's reagent) -

v) Rf - value - Quinine = 0.74 -, Cinchoninine = 0.66 -
- Quinidine = 0.62 -, Cinchonidine = 0.54 -

⑥ Spectrometric estimation - / HPLC (High performance -

i) Column -
ii) Solvent / mobile phase -
iii) Wave length -
iv) Flow rate -
v) Rt (Retention time) -

i) Column → Microsorb C_{18}

ii) Solvent/mobile phase → chloroform : Diethylamine -

iii) Wave length - 330nm -
iv) Flow rate - 1ml/minutes -
v) Rt → -

{→ine = Alkaloids}

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(c) HPTLC - [High performance thin layer chromatography]-

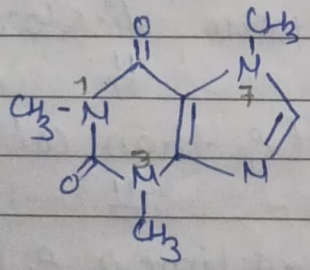
i> Plate -
ii> mobile/solvent phase -
iii> Wave length ⇒ 380nm -
iv> Rt -

i> Plate - Precoated silica gel $60F_{254}$ → [F = Fluorscence]

ii> mobile phase - Ethyl acetate : Diethylamine
iii> Flow rate - 1ml/minutes -
iv> Rt - 0.19 minutes -

Caffeine

1> Structure -



2> Chemico-taxonomical distribution -

• Caffeine is a purine alkaloids obtained from 'Tea leaves, coffee seeds, Cocoa & other species.-

* It consists of dried leaves of plant known as "Thea sinensis", family - "Theaceae"-

* It is chemically 1,3,7-trimethyl xanthine. It is isolated from tea & coffee seeds during "decaffeination process.-

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* Tea leaves contains 1-4% of caffeine & coffee contains 1-2% of caffeine.-
* It is white powder or white, glistering needle, odourless, bitter in taste, soluble in hot water.
* Caffeine is a CNS stimulant & diuretics.

3> # Extraction & Isolation-

- Take 20gm of tea leaves & add 10gm of Na_2Co_3 & water
⇓
- Boil for 20-25 minutes on heating mental & than filter it.
⇓
- Filtrate transfer into separating funnel & add 10 ml of chloroform / dichloromethane
⇓
- Agitation / shake it & than collect bottom transparent layer in beaker-
⇓
- Repeat it for 3-4 times & collect all transparent layer
⇓
- Transfer it into petridish & cover it with aluminium foil.
⇓
- After complete evaporation needle shape crystals of ~~coffe~~ caffeine obtaine. (white powder)
⇓
- It is recrystallized with alcohol-

4> # Characterisation / identification / chemical test / qualitative

i> Dragendroff's reagent test
ii> murexide test-

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* Murexide test -

· To the caffeine add HCl & potassium chlorate. ($KClO_3$) ⇓

· Heated to dryness - ⇓

· A purple colour is obtained by exposing the residue to vapours of dilute ammonia -

5) # Analysis / quantitative test -

a) TLC -

i) Sample preparation - Dissolve 1mg of caffein in 1ml of methanol / chloroform -

ii) Stationary phase - Silica gel-G -

iii) Solvent / mobile phase - Ethyl acetate : methanol : GAA [80 : 10 : 10]

iv) Spiring reagent - Expose to vapour of Iodine -

v) Rf - 0.41 -

b) HPLC - i) Column - microsorb C18 -

ii) mobile phase - water : methanol (70:30) -

iii) Flow rate - 1ml/minutes -

iv) Wave length - 275nm -

v) Rt - 2.6 minutes -

c) HPTLC - i) Plate - Pre coated silica gel 60f254 -

ii) Mobile phase - Ethyl acetate : methanol (80 : 20)

iii) Wave length - 275nm

iv) Rt - 0.39 minutes -

[C.E.-2022]

Reserpine

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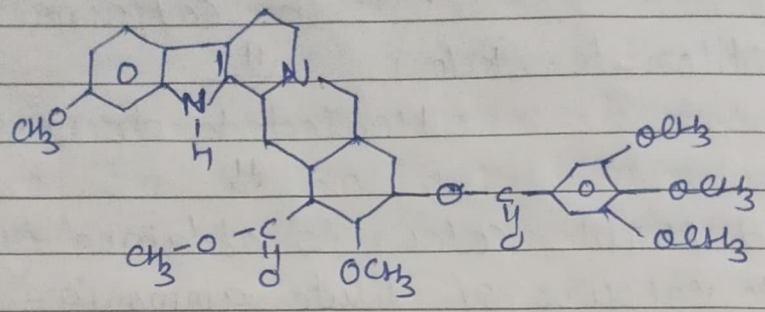
1) # Structure-



2) # Chemotaxonomical distribution-

- Reserpine is an indole alkaloid obtained from the roots of "Rauwolfia serpentina", family-Apocyano.
- It is a white or pale buff to slightly yellow, odourless, crystalline powder.-
- It is soluble in alcohol, acetone & chloroform.-
- Reserpine is an anti-hypertensive & anti-psychotic agents.

3) # Extraction & isolation -

- Rauwolfia root powder is exhaustively extracted with 90% alcohol by percolation -

⇓

- The alcoholic extract is conc. & dried under reduced pressure below 60°C to yield rauwolfia dry extract

⇓

- Rauwolfia ~~root~~ dry extract is extracted with Ether, chloroform & 90% alcohol (20:8:2.5) -

⇓

- Collect the extraction & add little dilute

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ammonia with intermittent shaking.- Add water & allow the drug to settle after vigorous shaking

⇓

- Filter off the solution & extract the residue with 4- volume of 0.5N ammonium sulphate in separating funnel. Combine all the extracts.-

⇓

- The extract is made alkaline with dilute ammonia to liberate alkaloid. Finally it is extracted with chloroform.

⇓

- Collect the chloroform extract, Conc. & evaporate on water bath to yield total rauwolfia alkaloids (30- different components)-

⇓

- Residue is subjected to column chromatographic fraction for the separation of reserpine.-

4) # Identification test -

i) Dragendroff's reagent test -

ii) Reserpine solution + dilute H_2SO_4 & expose to light

⇓

- It will give yellow colour with fluorscenee

iii) Sample solution + Vanillin in acidic acid

⇓

- Violet red colour is produce-

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5) # Analysis -

A) TLC - i) Solution p/sample preparation - Dissolve 1mg of reserpein in 1ml of methanol & chloroform. -

ii) Stationary phase - Silica gel-G -
iii) Solvent phase/mobile phase - Chloroform : Acetone : Di ethylamine (50 : 40 : 10) -
iv) Spiring reagent - Dragendroff's reagent -
v) Rf - 0.72

B) HPLC -
i) Column - Revers phase column -
ii) Flow rate - 1ml/minutes -
iii) Wave length - 254 nm -
iv) Rt - 21.68 minutes -
v) mobile phase - chloroform : Toluene : Ethyl acetate : Diethylamine (40 : 20 : 20 : 20)

C) HPTLC -
i) Plate - Precoated silica gel G-60 F_{254} -
ii) mobile phase - chloroform : Toluene : Ethyl acetate : Diethylamine (40 : 20 : 20 : 20)
iii) Wave length - 254nm
iv) Rt - 0.36 minutes -

ATROPINE

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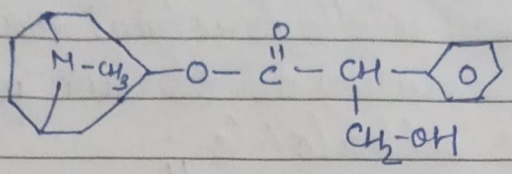
1.# structure-



2.# Chemotaxonomical distribution-

- Atropine is a tropane alkaloid.-
- It is obtained from "atropa belladona", datura stramonium & hyoscyamus niger, family- solanaceae
- It is a white crystal or crystalline powder & less soluble in water & highly soluble in organic solvents-
- It mainly act as CNS stimulents & depression action on nerve ending of secretary glands
- Injection of atropine is used in treatment of bradycardia.-

#3.# Extraction & isolation-

- Take weighed quantity of coarse powder & moisten with Na_2CO_3 solution ⇓
- Extract the blended mixture in petroleum ether. filter the petroleum ether extract-
⇓
- Extract the filtrate with aqueous acetic acid (alkaloids extracted in aqueous layer) -
⇓
- Extract the aqueous fraction with solvent ether & separate both fraction.* Discard solvent ether fraction.
⇓

⇓

* Aqueous (acidic fraction) made alkaline with Na_2CO_3 solution to obtain precipitates (ppt) of tropane alkaloids.-

⇓

- Filter the precipitate & dry to obtain residue.-

⇓

- Dissolve the residue in diethyl ether

⇓

- Filter & conc. the filtrate, atropine crystals will be separated out.-

4) # Identification test-

i) Dragendroff's reagent test-

** ii) Vitalin-morin test-

- Take small quantity of the solid atropine & add 2-drops of conc. nitric acid (HNO_3) in an evaporating dish & evaporated to dryness on water bath.-

⇓

- Then dissolve the residue in 1ml of acetone.-
- Add few drops of freshly prepared alcoholic potassium hydroxide solution-

⇓

- Violet colouration takes place due to tropane nucleus-

Analysis -

a) TLC -

i) Sample preparation - Dissolve 1mg of atropine in 1ml of organic solvents -

ii) Stationary phase - Silica gel - G -

iii) Solvent phase - Toluene : Ethylacetate : Diethylamine (70 : 20 : 10)

iv) Spiring reagent - Dragendroff's reagent

* Produce yellow-orange colour spot -

v) Rf - value - 0.7 -

b) HPLC - i) Column - C18

ii) Solvent/mobile phase - Ortho-phosphoric acid : Aceto-nitrile (70:30)

iii) Flow rate - 1.4 ml/minutes -

iv) Wave length - 215 nm -

v) Rt - 6.6 minutes -

c) HPTLC -

i) Plate - Precoated Silica gel - G - 60 F254 -

ii) Mobile phase - chloroform : methanol (70:30)

iii) Wave length - 200 nm -

iv) Rt - 0.42 minutes -

Resin

Curcumin

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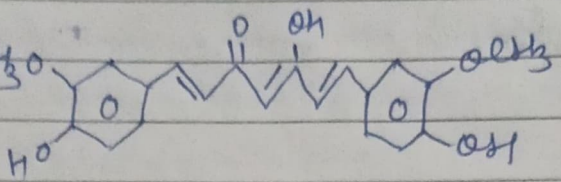
1> Structure-  [$C_{21}H_{20}O_6$] [Enol form]

2> Chemotaxonomical distribution-

· Curcumin or curcuminoids are the diaryl heptanoid compd. obtained from the dried rhizomes of turmeric "Curcuma longa", family- 'Zingiberaceae'

· Curcumin is the major colouring principle-
· It is a mixture of curcumin, mono-desmethoxy-curcumin & bis-des-methoxycurcumin.-
· It as an ~~organ~~ orange yellow, crystalline powder
· Insoluble in water & ether but soluble in alcohol.
· It is used as wound healing, anti-inflammatory, anti-arthritic & anti microbial activities-
· used against peptic ulcer.-

3> Extraction & Isolation ~~of~~

#

· Curcumin can be obtained by different process-

i> Turmeric powder is extracted with alcohol in soxhlet extractor. ⇓

· Alcoholic extract is concentrated under reduce pressure & dried-

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ii) Powder root is extracted with alcohol in soxhlation for 6 hrs.
⇓
- Collect the extract & again extract hexene
⇓
- Collect the hexene extract & re-extract with methanol
⇓
- Filter the solution & conc. on water bath to dryness
⇓
- To the residue add toluene & NaOH
⇓
- Collect ageous layer from the solution & add acid for gain pH 3.0 & made extract yellow
⇓
- Collect the extract & again extractated with diethyl ether
⇓
- Collect the ether layer wash with water & dryed over $MgSO_4$
⇓
- Yellow colour curcumine is often.-

#4) Identification test-

i) Powder drugs + H_2SO_4 ⇒ Crimson red colour

ii) Powder drugs + alkaline solution
⇓
Red-violet colour-

5.) Analysis -

(A) TLC -

i) Sample preparation → Dissolve 1mg curcumin in 1ml of methanol
ii) Stationary phase → Silica-gel-G-
iii) Solvent phase → Chloroform : Ethanol : Glacial acetic acid (94 : 5 : 1)
iv) Spiring reagent → Observed under U.V. light at 366 nm
v) Rf- value - 0.79 -

(B) HPLC -

i) Column - C18
ii) mobile phase - Acetonitrile : Acetic acid : water (80 : 10 : 10)
iii) Flow rate - 1ml/minutes -
iv) Wave length - 425 nm -
v) Rt - 7.04 minutes -

(C) HPTLC -

i) Plate - Precoated silica gel GF_{254} -
ii) mobile phase - Dichloromethan : methanol (50 : 50)
iii) ~~Wave~~ Wave length - 425 nm -
iv) Rt - 0.43 -

"Indian podophyllum" -
OR
Podophyllotoxin

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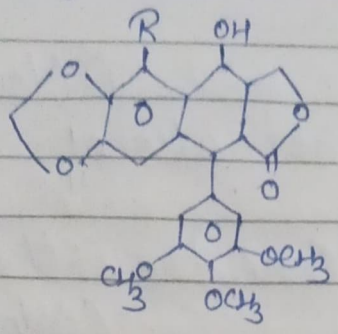
1. Structure-



R=H (Podophyllotoxin)
R=-OCH3 (5-methoxy-podophyllotoxin)

2. Chemotaxonomical distribution-

- It is also known as :
indian ~~phy~~ podophyllum -
* It is consists of the dried rhizome & root's of "Podophyllum emodi" (Indian), 'Podophyllum peltatum (American), family - "Berberidaceae"

* Podophyllum potent anti-cancer agents, anti-tumor agents, potent topical anti-viral & used in the HPV (Human papilloma-virus) infection & remove warts.-

3. Extraction & Isolation -

- 120gm root powder extract with 300ml methanol in soxhleter for 6hrs
⇓
- Filter the extract & filterate concentrate & add 200ml water containing 2ml HCl & cool it.-
⇓
- Allow mixture to stand for 2hrs below 5°C & filter it
⇓
- Filtrate was wash again with acidified water & filter it-
⇓

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- The residue after filtration is mix with sufficient quantity of hot alcohol -

⇓

- Filter it & Conc. the filtrate to get podophyllum - (Podophyllotoxin)

4> # Identification test -

- 0.5gm of the drug with 10ml of alcohol & filter

⇓

- Add 0.5ml copper acetate ⇒ Brown ppts produces
- Alkoholic extract + Add 5ml of 1M KOH ⇒ Stiff jelly is produce -

5>. # Analysis -

(A.) HPLC - i> Column = Reverse phase C18 -
ii> Mobile phase = methanol : water (60:40) -
iii> Wave length = 280nm -
iv> Flow rate = 0.9ml/min. -
v> Rt = 9.45 minutes -

(B.) HPTLC -
i> Plate → Precoated silica gel 60f254 -
ii> mobile phase = Acetonitrile : water (70 : 30)
iii> Wave length = 283 nm -
iv> Rt = 0.41 minutes -

#Glycosides

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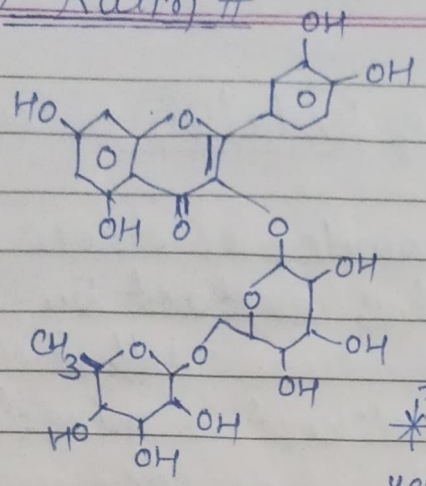
Rutin

1) # Structure-



Rutin (Rutoside) *

⇓

It is called vitamin P-

** * Pure rutin is yellow or yellow green color, needle shape crystal.

2) # Chemotaxonomical distribution-

- It is a citrus flavonoid glycoside containing drugs which mainly obtained from the root, rhizome, leaves part of the plants-

* The geneus of rutin it is very large consists of approximate to 550-750 species & is divided into 11-sub-genera-

* The rutin is mainly obtained from "phyllanthus amarus", "phyllanthus niruri", phyllanthum emblica", "phyllanthus urinaria"-

- family- Rutaceae

* Rutin is a pale yellow, needle shape structure, present in powder form.-

- It is tasteless & odourless-
- mainly soluble in formaldehyde, pyridine, methanol & alkaline solution-
- Rutin is mainly used as diuretic, bitter astrigents, liver, kidney & spleen problem & also act as anti-septic-

** Rutin is also known as "Bhui-amla"-

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3> # Extraction & Isolation -

- Take 20gm powder of drugs & add 250ml of 80% ethanol & extract by soxhletion method
⇓
- Filter the extract & mixed with 25ml of water
⇓
- Again extracted with petrolium ether & chloroform
⇓
- Take aq. layer & keep it cold for 72 hrs & yellow ppt was separated.-
⇓
- Yellow ppt was wash with chloroform : ethyl acetate : ethanol (50 : 25 : 25)
⇓
- After washing ppt is dissolve in hot methanol & filter it
⇓
- The filtrate it cone. to dryness -
⇓
- Yellow needle shape crystals are obtain - (Rutin)

4> # Identification test -

i> Drugs with ferric ⇒ Give dark green color -
ii> Drugs with lead acetate ⇒ Orange yellow ppt -
iii> Drugs with ammonium molybdate & antimony trichloride ⇒ orange - yellow ppts -
iv> Shinoda test - few fragments of mg - ribbon + Cone. HCl & add ethanolic extract
⇓
Red to pink colour -

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Analysis -

(A) HPLC -

i) Column - Reverse phase C_{18} -
ii) mobile phase - methanol : water (70 : 30) -
iii) Wave length - 280 nm -
iv) Flow rate - 0.5 ml/min -
v) Rt - 0.5 minutes -

(B) HPTLC -

i) plate - precoated silica gel 60f254 -
ii) mobile phase - Acetonitrile : water (60 : 40) -
iii) Wave length - 254 nm -
iv) Flow rate - 5 ml/minutes -
v) Rt - 0.65 minutes -

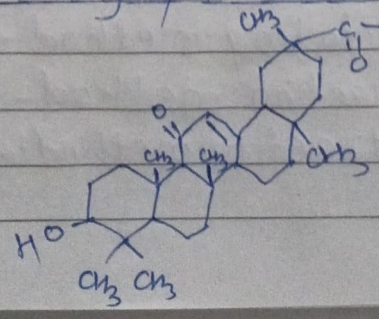
(C) IR - value / IR - spectroscopy -

functional group	Peak
-OH	3330
-C=O	1660
-CH	2920
-C=C	1600
-C-O-C	1360

Exam -

Glycyrrhetinic acid # (5)

1) # Structure :-



$C_{30}H_{46}O_4$

* Glycyrrhizin is 30-50 times as sweet as sucrose.
* Pure glycyrrhizin is odorless.

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2) # Chemotaxonomical distribution -

· Glycyrrhetinic acid is mainly obtain from sweet wood, Liquorice & multhi, yastimadhu.

* It is obtained from the dried roots & stolons of "Glycyrrhiza glabra", family - Leguminosae.
* Chief constituent of liquorice is "glycyrrhizin.

* Glycyrrhizin -

· A triterpenoid saponin glycoside.

· Also called as 'glycyrrhizic Ā or glycyrrhizinic acid.

* Being a glycoside, glycyrrhizic acid on hydrolysis given an aglycone & a glycone portions

⇒ Glycone = Glycyrrhizinic acid.
⇒ Aglycone = triterpenoidal structure of glucuronic Ā.

3) # Extraction & Isolation

· The isolation of glycerrhizin from glycerrhiza is based on solubility.

· The three (3) method of isolation are -
a) Acid precipitating method.
b) Alcohol extraction method.
c) Ammonia extraction method.

a. Acid precipitating method -

- Weigh liquorice powder & add water in the beaker & boil with continuous stirring -
- Decant/filter the supernant liquid
- filter the remaing residue & collect the filterate
- Adjust pH 2.8 by the addition of acid -
- Glycerrhizin precipitates out -
- filter & collect the glycerrhizin ppt -
- Wash the ppt with cold water to make it free from acid
- Transfer the ppt to china dish & heat gently to remove the water content, shiny brown mass of glycerrhizin is seen. -

(b.) Alcohol extraction method -

- Weigh liquorice powder & add 100ml methanol in 500ml beaker & properaly mix it
- Add another 100ml methnol & left for 24 hrs -
- filter & collect the filtrate -

⇓

- Extract this methanolic extract with 3-portions of petroleum ether, subsequently with benzene, ethylacetate chloroform & solvent ether.

⇓ filter

- Transfer methnolic layer into china dish & evaporate on water bath to get glycerrhizin.

c) Ammonium extraction method-

- Glycerrhiza is extracted with hot water & filtered
- ⇓
- filtrate is acidified with Conc. H_2SO_4 & pH 2.8
- ⇓
- ppt is dissolved in dil. NH_4OH & poured into acetone to ppt ammonium glycerrhizinate.
- ⇓
- The ppt is dissolved in hot water & evaporated to get ammonium glycerrhizinate-

4) # Identification test-

i) Foam test-

ii) Haemolysis test-

iii) Libermann test →

- Drugs solution + Acet. anhydride
- ⇓ Boil
- Add H_2SO_4 ——— ▷ Blue colour appears-

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5.) # Analysis -

a) TLC -

i) Sample preparation - Dissolve 1mg of liquorice in 1ml of alcohol

ii) Stationary phase - Silica gel-G -

iii) mobile phase - ~~Form~~ Ethyl acetate : formic acid : GAA (15:1:2)

- Butanol : GAA : water (7:1:2) -

iv) Spiring reagent - Libermann-reagent

- Vapour of Iodine -

v) R_f - 0.69 -

b) HPLC - i) Column - Reverse phase C18 -

ii) Spiring reagent - photo-diode array diter

iii) mobile phase - Acetonitrile : phosphoric acid (80:20) -

iv) Wave length - 230nm -

v) flow rate - 0.6ml/minutes -

vi) Rt - 0.6 minutes -

c) HPTLC - i) Plate - Precoated silica gel 60f254 :

ii) mobile phase - Ethyl acetate : Ethanol : water : Ammonia (60:20:10:10)

iii) Wave length - 254nm -

iv) Rt - 0.42 minutes -

Artemisin

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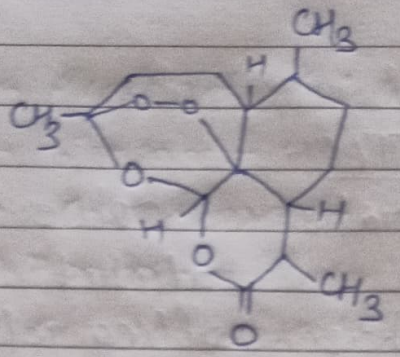
1) # Structure -



2) # Chemotaxonomical distribution -

- Artemisin is a ses-quiterpenoid lactone. Obtained from the flower, head of part of the plant "Artemisia annua", "Artemisia citro". family - Compositae/Asteraceae

* synonyms of artimisin is "Santonica" -
* Artimisin is a white crystalline powder. Soluble in organic solvents & insoluble in water -
* Artemisin is an anti-malarial drugs which used in the treatment of malarial & many other disease.

3) # Extraction & Isolation -

- The leaves are air dried, coarsely powdered & extracted with petroleum ether

⇓

- The extract is conc., dried & re-dissolved in chloroform. Add acetonitrile to ppt sugar & waxes -

⇓

- Filter & collect the filtrate. Evaporate to dryness to yield residue. -

⇓

- The chromatographic fractionation of the conc. on silica gel by eluting with chloroform : ethyl acetat

* Primary solvent ⇒ "Petroleum ether" → Because remove the impurity from ~~drugs~~.

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yields the fraction of artemisin. -

⇓

- The fraction containing artemesin could by crystalline from cyclohexane or 50% ethanol. -

4) # Identification test -

- Boil 1gm finely powdered drug with 10ml alcohol & filter.

⇓

- To the filtrate, add NaOH & heat again. -

⇓

- The liquid develops red colour -

⇓

- Indigate the presence of artemisin. -

5) # Analysis -

(A) T.L.C. - i) Sample preparation - Dissolved 1mg of artemisin in 1ml of chloroform -
ii) Stationary phase - Silica gel-G -
iii) Mobile phase - Petroleum ether : Ethyl acetate (5:5) -
iv) Spraying reagent - Para-dimethyl-amino-benzaldehyde & at 80°C to produce colour -
v) Rf value - Compare with standard artemisin -

(B) HPLC -
i) Column - Reversephase C18 -

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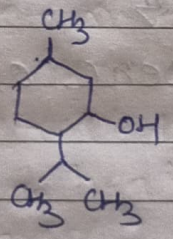
ii) mobile phase - Acetonitrile : water (80:20) -
iii) wave length - 250 nm -
iv) Flow rate - 0.5ml/min. -
v) Rt - 0.7 min -

(c) HPTLC -
i) Plate - Precoated silica gel $60F_{254}$ -
ii) mobile phase - n-hexane : Ethyl acetate (70:30) -
iii) wave length - 540 nm -
iv) Rt - 0.7 min. -

Menthol

1) # Structure -



2) # Chemotaxonomical distribution -

• menthol is an organic compd., belong to terpenoids group -
* menthol is mainly obtained from leaves part of the plant "Mentha piperita", Mentha spicata", family - 'Labiatae' -
* menthol is a waxy, crystalline, clear substance or white in colour -
** menthol is solid at room temperature. -
* menthol is a mono-terpene alcohol obtained from different variety of mint oils or peppermint oils. -

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* Freely soluble in organic solvents like alcohol, chloroform, Carbon tetrachloride, ether & other solvent -
* menthol is mainly used as local anesthetic & counter irritent. -
* menthol also used in throat irritetion & dental care as a topical anti-bacterial agents-

Extraction & Isolation-

- Take the accurately weighed quantity of coarse powder of mentha piperita parts just before flowering
⇓
- Extract the peppermint oil by water distillation method by the help of clevenger apparatus-
⇓
- Separate the oil & allow cooling. Crystals of (-) methol will separate out. -
⇓
- Collect the crystals by "Centrifugation" -
⇓
- Re-crystallize menthol from acetone or any other low boiling point solvent. -

Identification test-

- few gm of menthol + few drops of Conc. H_2SO_4 + few drops ~~vatti~~ Vanillin sulfuric acid reagent
⇓
- Orange colour apper ⇒ when add water in orange colour ppt ⇒ orange change violet

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* Small piece of KOH added into test tube containing 1ml oil, then heated

↓

- Cool the solution & add 1ml of diethyl ether & few drops of carbon disulfide

↓

- Yellow residue is obtain -

5) # Analysis -

(A) T.L.C. - i) Sample preparation -
- Dissolved 1mg of menthol in 1ml of menthanol -

ii) Stationary phase - Silica gel-G -

iii) Mobile phase - Pure chloroform -

iv) Spraying reagent - 1% Vanillin-Sulphuric acid reagent & heat the plate 110°C for 10minutes -

v) Rf-value - 0.48 - 0.62 -

(B) HPLC -

i) Column - Shim pack VP-ODS -

ii) Column temp. - 25°C

iii) Mobile phase - Pure ethyl acetate -

iv) Wave length - 322 nm -

v) Rt → 0.4min. -

Citral

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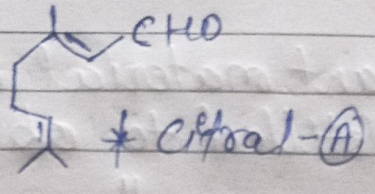
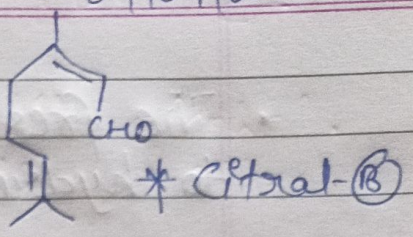
1) # Structure-



* Citral-(A)



* Citral-(B)

2) # Chemotaxonomical distribution-

· Citral is also known as "Lemonal"

* Citral is monoterpine aldehyde found in variety of sources like- lemon grass, lemon peels & orange peels etc.
* Citral is mainly obtain from the lemon grass "Cymbopogon flexuosus", ·family- Graminae-
* 75-85% of citral present in lemon grass-
** Citral have -2- Isomers-
i) E-Isomers is known as "Geranial | Citral-(A)"-
ii) Z- Isomers is known as "Neral | Citral-(B)"-

* It is yellow colour oil mainly soluble in organic solvent & insoluble in water.-
** Geranial & Neral both are light oily liquids with lemon odour-
* Citral is mainly used in perfume, flavouring agents & many other chemical industry.-
** Citral also have significe production of Vit. A-
* Citral has strong anti-microbial properties-
* It has an effect on insect & act as mild repellent for some species.-

3) # Extraction & Isolation-

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- The fresh plant material is collected & subjected to hydro-distilled to obtain lemon grass oil
⇓
- Purification of lemon grass oil is perform by fractional crystallization -
⇓
- To the total oil, first sodium sulphite is added, the citral get converted into its sulphite salt -
⇓
- The salt crystallizes out of the solution -
⇓
- The crystals are filtered & washed with ether or chloroform. -
⇓
- The product is then subjected to sodium carbonate treatment to recover citral -

4) # Identification test -

i) Sudan red III -
- Take a thin section of drug & add alcoholic solution of sudan red III
⇓
- Red colour, which indicate presence of volatile oil.

ii) Tincture alkana -
- Take thin section of the drug & add a drop of tincture alkana -
⇓
- Red colour is produced which indicates presence of volatile oil -

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Analysis -

(A) T.L.C.

i) Sample preparation - Dissolve 1mg of citral in 1ml of ~~menthol~~ - methanol.

ii) Stationary phase - Silica gel - G.

iii) Mobile phase - Pure chloroform -

iv) Spiring reagent - 2,4 - dinitrophenyl hydrazine reagent

v) Colour - Yellow to orange -

vi) Rf value - 0.51 -

(B) HPLC - i) Column - ODs - hypersill column -

ii) Mobile phase - Methanol -

iii) Flow rate - 1ml/minutes -

iv) Wave length - 254 nm -

(C) HPTLC -

i) Plate - Pre coated Silica gel 60f254 -

ii) Mobile phase - Pure toluene -

iii) Wave length - 595 nm -

iv) Flow rate - 1ml/min. -

v) Rt - 0.7min. -

** Screening speed = 20mm/sec. -